Clinical impact of virological failure and resistance analysis definitions used in pivotal clinical trials of initial antiretroviral treatment: a systematic review.

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Hortensia Álvarez[1], Miguel Yzusqui[2], Josep M Llibre[3]
[1]Infectious Diseases Unit, Internal Medicine Department, University Hospital of Ferrol, A Coruña, Spain.
[2]Internal Medicine Department, Hospital Nuestra Señora del Prado, Talavera de la Reina, Toledo, Spain
[3]Infectious Diseases and "Fight AIDS" Foundation, University Hospital Germans Trias i Pujol, Badalona, Barcelona, Spain

Hortensia Álvarez
hortensia.alvarez.diaz@sergas.es

Background and Aim.

There are no standardised criteria for defining confirmed protocol-defined virological failure (PDVF) nor criteria for inclusion into the resistance analysis population (RAP) in phase III randomised clinical trials (RCT) of initial antiretroviral therapy (ART). Guidelines define VF as a confirmed viral load (VL) >200 copies/ml, a threshold that eliminates most cases of apparent viremia caused by VL blips or assay variability.[1] The choice of higher thresholds of HIV-1 RNA to perform a genotyping is usually attributed to the lack of validation of these FDA-approved tests in participants with <1000 copies/mL and the possibility of rendering inaccurate genotypic results with lower VLs.[2] Recent analyses have shown a valid genotype amplification with results predictive of future virological outcomes in samples with HIV-1 RNA 51-199 copies/mL.[3,4]
We assessed the **clinical impact of mismatching between virological non-response (HIV-1 RNA ≥ 50 copies/mL), confirmed PDVF and RAP definition in studies with the newest first-line ART, at 48 weeks**.

Methods.

Systematic review of all phase III RCTs[5-18] including preferred once-daily ART (European AIDS guidelines)[19] or recently approved by the FDA, according PRISMA guidelines.[20]

Results.

-**16 Treatment arms** (14 RCTs) with 6,175 participants treated with dolutegravir (DTG), bictegravir (BIC), elvitegravir/cobicistat (EVG/c), raltegravir (RAL), darunavir/cobicistat (DRV/c), rilpivirine (RPV) or doravirine (DOR)(**Table 1**).
-**Plasma HIV-1 RNA thresholds for PDVF or RAP** ranged from 40 to 50, 200, 400 and 500 copies/mL (**Table 2**) .
-**Only eight treatment arms genotyped all participants with PDVF**. Most of the remaining eight arms genotyped roughly ≤50% of those with PDVF.
Overall, 85/296 (29%) patients with PDVF were not genotyped. We found a strong evidence of a linear correlation between the higher HIV-1 RNA threshold for genotyping and increasing rates of participants with PDVF that were not eventually genotyped (**Table 3**).
-**No resistance was selected against the third drug or the backbone nucleos(t)ide reverse transcriptase inhibitors (NRTIs) in any participant in the studies with DTG, BIC or DRV/c**. EVG/c, RAL and RPV, showed selection of HIV-1 resistance against both the third drug and the NRTIs used in the backbone in approximately 50% of the participants with PDVF and genotypes successfully performed.
-Percentages of participants with drug resistance mutations selected at VF, and participants meeting PDVF criteria but with no genotype data available (not genotyped for HIV resistance or failed amplification) at 48 weeks, are shown in **Table 4 and Figure 1**.

Table 1. Week 48 phase III clinical trials of the newest once-daily antiretroviral drugs in first-line therapy in participants with HIV-1 infection: main characteristics.

Third drug	Clinical Trial	Design	Arm size (n)	Female (%)	NRTIs	Comparator arm	High VL (>10^5 c/mL) (%)	Low CD4 (<200 cells/µL)(%)	Efficacy: VL <50c/mL at 48 weeks % (95% CI) ♦
DTG	SINGLE [5]	DB	414	16·0	3TC/ABC	EFV	32·0	14·0	88% vs 81%; 7 (2 to 12)
	SPRING-2 [6]	DB	411	15·0	2NRTIs	RAL	28·0	13·0	88% vs 85%; 2·5 (-2·2 to 7·1)
	FLAMINGO[7]	OL	242	13·0	2NRTIs	DRV/r	25·0	10·0	90% vs 83%; 7·1 (0·9 to 13·2)
	‡ARIA [8]	OL	248	100·0	3TC/ABC	ATV/r	28·0	26·0	82% vs 71%; 10·5 (3·1 to 17·8)
	‡GS-US-380-1489 [9]	DB	315	10·0	3TC/ABC	BIC/FTC/TAF	16·0	10·0	93% vs 92%; -0·6 (3·6 to -4·8)
	GS-US-380-1490 [10]	DB	325	11·0	FTC/TAF	BIC	17·0	10·0	93% vs 89%; -3·5 (1 to -7·9)
BIC	‡GS-US-380- 1489 [9]	DB	314	9·0	FTC/TAF	DTG/3TC/ABC	17·0	11·0	92% vs 93%; -0·6 (-4·8 to 3·6)
	‡GS-US-380-1490 [10]	DB	320	13·0	FTC/TAF	DTG	21·0	14·0	89% vs 93%; -3·5 (-7·9 to 1)
EVG/c	‡GS-US-292-0104 and 0111 [11]	DB	866	15·0	FTC/TAF	FTC/TDF	23·0	13·0	92% vs 90%; 2 (-0·7 to 4·7)
RAL QD	ONCEMRK [12]	DB	531	17·0	FTC/TDF	RAL 400 mg BID	28·0	13·0	90% vs 90% ; -0·4 (-4·9 to 4)
DRV/c	‡AMBER [13]	DB	362	12·0	FTC/TAF	FTC/TDF	16·6	6·1	91% vs 88%; 2·7 (-1·6 to 7·1)
RPV	ECHO [14]	DB	346	23·0	FTC/TDF	EFV	48·0	33·0Φ	83% vs 83%; -0·4 (-5·9 to 5·2)♦♦
	THRIVE [15]	DB	340	26·0	2NRTIs	EFV	45·0	33·0Φ	86% vs 82%; 3·9 (-1·6 to 9·5)♦♦
	‡STaR [16]	OL	394	7·0	FTC/TDF	EFV	34·0	13·0	86% vs 82%; 4·1 (-1·1 to 9·2)
DOR	‡DRIVE-AHEAD [17]	DB	364	16·0	3TC/TDF	EFV	20·0	12·0	84% vs 81%; 3·5 (-2 to 9)
	DRIVE-FORWARD[18]	DB	383	17·0	2NRTIs	DRV/r	22·0	11·0	84% vs 80%; 3·9 (-1·6 to 9·4)

ABC, abacavir; 3TC, lamivudine; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide;; DB, Double –blind; OL, Open-label
‡ Fixed dose combination (FDC) Φ % of participants with CD4 < 200 cells/µl in pooled ECHO & THRIVE data ♦ Efficacy of the study arm *vs* control arm; adjusted treatment difference, 95% confidence interval (CI). Intention-to-treat (ITT) US FDA-defined snapshot algorithm unless otherwise specified ♦♦ Intention-to-treat- time- to-loss of virological response (ITT-TLOVR)

Figure 1. Percentages of participants with drug resistance mutations selected at virological failure, and participants meeting PDVF criteria but with no genotype data available at 48 weeks.

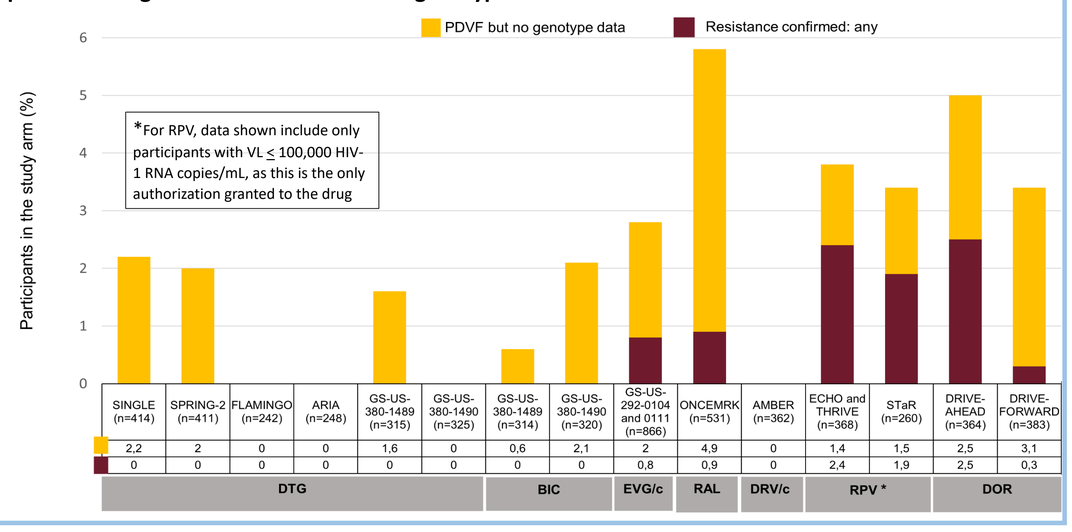

Table 2. Definition rules for virological failure and resistance testing at week 48.

Third drug	Clinical Trial (n)	VF definition (PDVF)	Criteria for resistance testing	Sample for resistance
DTG	SINGLE (n=414)[5]	2 VL ≥ 50 c/mL on or after week 24	2 VL ≥ 50 c/mL on or after week 24	First
	SPRING-2 (n=411) [6]	2 VL ≥ 50 c/mL on or after week 24	2 VL ≥ 50 c/mL on or after week 24	First
	FLAMINGO (n=242) [7]	2 VL > 200 c/mL on or after week 24	2 VL > 200 c/mL after week 24	First
	ARIA (n=248)[8]	2 VL ≥ 400 c/mL on or after week 24*	2 VL ≥ 400 c/mL on or after week 24	First
	GS-US-380-1489 (n=315)[9]	Confirmed virological rebound ≥ 50 c/mL or last available HIV-1 RNA ≥ 50 c/mL	2 VL ≥ 50 c/mL with the second VL ≥ 200 c/mL or ≥ 200 c/mL at week 48 or last study visit	Second
	GS-US-380-1490 (n=325)[10]	Confirmed virological rebound ≥ 50 c/mL or last available HIV-1 RNA ≥ 50 c/mL	2 VL ≥ 50 c/mL with the second VL ≥ 200 c/mL or ≥ 200 c/mL at week 48 or last study visit	Second
BIC	GS-US-380-1489 (n=314)[9]	Confirmed virological rebound ≥ 50 c/mL or last available HIV-1 RNA ≥ 50 c/mL	2 VL ≥ 50 c/mL with the second VL ≥ 200 c/mL or ≥ 200 c/mL at week 48 or last study visit	Second
	GS-US-380-1490 (n=320)[10]	Confirmed virological rebound ≥ 50 c/mL or last available HIV-1 RNA ≥ 50 c/mL	2 VL ≥ 50 c/mL with the second VL ≥ 200 c/mL or ≥ 200 c/mL at week 48 or last study visit	Second
EVG/c	GS-US-292-0104 and 0111 (n=866)[11]	VL ≥ 50 c/mL and < 1 $\log_{10}$ reduction from baseline at week 8, or VL ≥ 50 c/mL after previous suppression to < 50 c/ mL or >1 $\log_{10}$ increase from nadir	2 VL ≥ 50 c/mL after achieving < 50 c/mL and the second VL ≥ 400 c/mL; or VL ≥ 400 c/mL at week 48 or last study visit	Second
RAL QD	ONCEMRK (n=531)[12]	VL ≥ 40 c/mL by week 24 or 2VL ≥ 40 c/mL after initial < 40 c/mL	VL ≥ 500 c/mL	Second
DRV/c	AMBER (n=362)[13]	Confirmed < 1$\log_{10}$ VL reduction from baseline and VL ≥ 50 c/mL at week 8 , or VL ≥ 50 c/mL after previous suppression to < 50 c/mL or > 1$\log_{10}$ VL increase from nadir or VL ≥ 400 c/mL at endpoint or last study visit after week 8	VL ≥ 400 c/mL	Any†
RPV	ECHO (n=346)[14]	Never achieved 2VL < 50 c/mL and ≥ 0·5 $\log_{10}$ above nadir or 2 VL ≥ 50 c/mL after 2VL < 50 c/mL (or single, when last available)	Never achieved 2VL < 50 c/mL and ≥ 0·5 $\log_{10}$ above nadir or 2 VL ≥ 50 c/mL after 2VL < 50 c/mL (or single, when last available)	First
	THRIVE (n=340)[15]	Never achieved 2VL < 50 c/mL and ≥ 0·5 $\log_{10}$ above nadir or 2 VL ≥ 50 c/mL after 2VL < 50 c/mL (or single, when last available)	Never achieved 2VL < 50 c/mL and ≥ 0·5 $\log_{10}$ above nadir or 2 VL ≥ 50 c/mL after 2VL < 50 c/mL (or single, when last available)	First
	STaR (n=394)[16]	VL ≥ 50 c/mL and < 1 $\log_{10}$ reduction from baseline at week 8, or VL ≥ 50 c/mL after previous suppression to < 50 c/ mL, or >1 $\log_{10}$ increase from nadir	VL ≥ 400 c/mL at week 48 or last study visit (at or after week 8) or suboptimal virological response (less than 1 $\log_{10}$ decrease in VL from baseline at week 8 and confirmed at the subsequent visit) or confirmed VF	Second
DOR	DRIVE-AHEAD (n=364)[17]	Confirmed VL ≥ 200 c/mL at week 24 or week 36 or confirmed VL ≥ 50 c/mL at week 48 or confirmed VL ≥ 50 c/mL after initial VL< 50 c/mL	VL > 400 c/mL	Any††
	DRIVE-FORWARD (n=383)[18]	Confirmed VL ≥ 200 c/mL at week 24 or week 36 or confirmed VL ≥ 50 c/mL at week 48 or confirmed VL ≥ 50 c/mL after initial VL < 50 c/mL	VL > 400 c/mL	Second

*VF not defined throughout ARIA study. Instead, criteria for virological withdrawal were defined as specified above †PDVF with VL ≥ 400 c/mL at failure (preferably confirmed, or otherwise at unconfirmed VF timepoint) or at later timepoints ††Both samples (first and second) or either one, if VL > 400 c/mL

Table 3. Cochran-Armitage trend test.

n= participants not genotyped
N=participants with PDVF

VL threshold for genotyping	n	N	%	p
50	0	110	0,0	<0,001
200	13	32	40,6	
400	50	118	42,4	
500	22	36	61,1	
Total	85	296	28,7	

Table 4. Main virological outcomes and HIV resistance analysis findings at week 48.

Third drug	Clinical Trial (n)	Virological non-response, VL≥ 50c/mL; n (%)*	Confirmed VL>200 c/mL; n (%)	PDVF n (%)	Met criteria for inclusion in RAP, n(%)	Met PDVF definition but not included in RAP, n (%)	Failed amplification n (%)	Emergent resistance: Any, n (%)	Emergent resistance:Third drug, n (%)	Emergent resistance: NRTIs, n (%)
DTG	SINGLE (n=414)[5]	21 (5·1)	2 (0·5)	18 (4·3)	18 (4·3)	0	9 (2·2)	0	0	0
	SPRING-2 (n=411)[6]	20 (4·9)	7 (1·7)	20 (4·9)	20 (4·9)	0	8 (2·0)	0	0	0
	FLAMINGO (n=242)[7]	15 (6·2)	2 (0·8)	2 (0·8)	2 (0·8)	0	0	0	0	0
	ARIA (n=248)[8]	16 (6·4)	NA	6 (2·4)	6 (2·4)	0	0	0	0	0
	GS-US-380-1489 (n=315)[9]	8 (2·5)	4 (1·2)	8 (2·5)	4 (1·3)	4 (1·3)	1 (0·3)	0	0	0
	GS-US-380-1490 (n=325)[10]	4 (1·2)	NA	4 (1·2)	5 (1·5)†	NA	0	0	0	0
BIC	GS-US-380-1489 (n=314)[9]	3 (1·0)	1 (0·3)	3 (1·0)	1(0·3)	2 (0·6)	0	0	0	0
	GS-US-380-1490 (n=320)[10]	14 (4·4)	7 (2·1)	14 (4·4)	7 (2·1)	7 (2·1)	0	0	0	0
EVG/c	GS-US-292-0104 and 0111 (n=866)[11]	31 (3·6)	NA	31 (3·6)	19 (2·2) ††	17 (2·0)	0	7 (0·8)	5 (0·5)&	7 (0·8)&&
RAL QD	ONCEMRK (n=531)[12]	29 (5·5)**	6 (1·1)	36 (6·8)	14 (2·6)	22 (4·1)	4 (0·7)	5 (0·9)	4 (0·7)&	5 (0·9)&&
DRV/c	AMBER (n=362)[13]	16 (4·4)	8 (2·2)	8 (2·2)	8 (2·2)‡	0	0	0	0	0 &&&
RPV	ECHO (n=346)[14]	38 (11·0)***	NA	45 (13·0)	45 (13)	0	5 (1·4)	29 (8·3)	26 (7·5) ♦	28 (8) ♦♦
	THRIVE (n=340)[15]	24 (7·1)***	NA	27 (7·9)	27 (8)	0	5 (1·5)	15 (4·4)	13 (3·8) ♦	14 (4·1) ♦♦
	STaR (n=394)[16]	32 (8·1)	NA	32 (8·1)	20 (5·1)	12 (3·0)	0	17 (4·3) Φ	16 (4·1) ♦	16 (4·1) ♦♦
DOR	DRIVE-AHEAD (n=364)[17]	39 (10·7)	12 (3·3)	22 (6·0)	13 (3·6)‡‡	9 (2·5)	1 (0·2)	9 (2·5)	7 (1·9) ♦	9 (2·5) ♦♦
	DRIVE-FORWARD (n=383)[18]	43 (11·2)	7 (1·8)	19 (5·0)	7 (1·8)‡‡‡	12 (3·1)	1 (0·3)	1 (0·3)£	1 (0·3)	1 (0·3)

NA, not available *TT US FDA-defined snapshot algorithm unless otherwise specified **Analysis done by VF snapshot algorithm, defined as VL≥ 40 copies/mL at week 48 *** ITT- Time-to-loss of virological response (TLOVR) & Integrase emergent mutations (no. of participants): GS-US-292-0104 and 0111 studies: T66A (1), E92Q (2), Q148R+ T66I/A (1), N155H (1); ONCEMRK study: L74M + E92Q (1), N155H (1), V151I+ N155H (1), N155H+ I203M (1) && NRTI emergent mutations (no. of participants): GS-US-292-0104 and 0111 studies: M184V/I (6), M184V/I+ K65R (1); ONCEMRK study: M184V (3), M184M/I/V (1), V118I+ M184M/I/V (1) &&& M184I/V was detected pre-treatment by deep sequencing as a minority variant (9.4%) † One participant without PDVF was included in RAP ††5 participants who re-suppressed HIV-1 RNA to < 50 copies/mL while maintaining study drugs, were not genotyped· Only 14 participants were genotyped ‡One participant was released after database lock and was re-suppressed at following unscheduled visit, so was missing for the Week 48 analyses. Out of the 8 PDVF, 2 showed response and 6 VF as defined by FDA snapshot ‡‡13 out of 22 participants meeting PDVF and 10 out of 35 participants discontinuing for reasons other than PDVF, were included in RAP. In addition, 5 participants included in RAP were not tested for resistance for various reasons (protocol deviation or lost to follow-up) ‡‡‡ 7 out of 19 participants meeting PDVF and 2 out of 40 participants discontinuing without PDVF, were included in RAP ♦Most frequent NNRTI emergent mutations (no. of participants): ECHO study: E138K (18), K101E (5), Y181C (5); THRIVE study: E138K (10), K101E (3), V189I (2); STaR study: Y181C/I (8), E138K/Q (6), K101E (5); DRIVE-AHEAD study: V106I/V/A/M/T (4), F227C/R (4) ♦♦Most frequent NRTI emergent mutations (no. of participants): ECHO study: M184V/I (26), K65R (3); THRIVE study: M184V/I (12); STaR study: M184V/I (15), K65R/N (3); DRIVE-AHEAD study: M184V (4), K65R/K (2) ΦAny emergent third drug or NRTI resistance by baseline HIV-1 RNA: 5/260 in VL ≤ 100.000c/mL, 5/98 in VL >100.000-500.000c/mL, 7/36 in VL >500.000c/mL £One of the 2 participants who discontinued at Week 24 without PDVF, developed resistances against DOR (V106I, H221Y, F227C) and FTC (M184V).

Conclusions.

1-The absence of standardised definitions of VF and criteria for resistance testing in pivotal phase III RCTs of first-line ART leads to the possibility of underreporting of resistance mutations when genotypes are only performed at higher viral load cut-offs.

2-Stringent homogeneous criteria should be defined to ensure that all participants with PDVF (confirmed HIV RNA ≥ 50 copies/mL and the second >200 copies/mL) undergo genotyping.

Funding. The authors received no specific funding for this work.

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